Amendments to the Specification

Please replace paragraph [0003] at page 2 with the following paragraph:

Several commercial kits are available for the rapid extraction of proteins from cells. Two of the most popular are ~~BugBuster~~[[™]] BUG BUSTER® (Novagen, detergent containing Complete Protease Inhibitor Cocktain Tablets (Roche)) and B-PER® (Pierce, lysis buffer containing mild nonionic detergent in 20 mM Tris HCl, pH 7.5). Both of these kits employ the use of a detergent solution to disrupt the cell membrane, thereby releasing the cellular components including protein. Neither of these methods couple a purification step with the extraction method. The ~~BugBuster~~[[™]] BUG BUSTER® (detergent containing Complete Protease Inhibitor Cocktain Tablets (Roche)) method utilizes a ~~Benzonase~~BENZONASE® nuclease (recombinant endonuclease) to decrease the viscosity in the lysate due to the large amounts of chromosomal DNA present in the sample after lysis. However, the product does not include any method for removal of the small DNA fragments which are necessarily generated by the nuclease digestion. The B-PER® product (lysis buffer containing mild nonionic detergent in 20 mM Tris HCl, pH 7.5) is solely intended as an extraction system. The system includes a centrifugation step, which removes some insoluble debris; however, there is no subsequent purification. Any contamination of the lysates generated with the B-PER® product (lysis buffer containing mild nonionic detergent in 20 mM Tris HCl, pH 7.5) must be removed using separate methods of purification.

Please replace paragraph [0014] at pages 6-7 with the following paragraph:

According to the invention, the matrix may be any porous material that retards the flow of high molecular weight molecules, structures and aggregates, and/or does not substantially retard the flow of low molecular weight molecules. Such matrices may include but are not limited to a polyester matrix, a polyolefin matrix, a scintered polyethylene matrix, a nitrocellulose matrix, a cellulose acetate matrix, a cellulose matrix, a porous ceramic matrix, a silica matrix, a polysaccharide matrix (SEPHAROSE™ (cross-linked agarose), agarose, SEPHADEX® (cross-linked dextrans), etc.), a polymer matrix (SEPHACRYL™ (covalently-linked acryldextrans), TRISACRYL (copolymers of N-[tris(hydroxymethyl) methyl] acrylamide), TOYOPEARL® (polyvinyl alcohol gel), BIO-GEL® (activated polyacrylamide gel), etc.) and the like. In a preferred aspect, the matrix is a solid matrix, although the matrix may be a semi-solid matrix. Suitable matrix materials may be obtained commercially, for example from Filtrona Richmond, Inc. (Richmond, Virginia), Bio-Rad (Richmond, California), Gentra Systems (Minneapolis, MN), Tosohaas (Montgomeryville, PA), BioSepra, Inc., (Marlborough, MA), and Porex Technologies Corp. (Fairburn, GA). In a related aspect, the matrix may be prepared in various sizes, shapes, and forms including flat, wafer, cylindrical, rectangular, beads, gels, square, cartridge, swab tip, plug, frit, membrane and the like, and may also be contained in various containers such as tubes, bottles, vials, ampules, microspin tubes, wells, multi-well plates, bags and the like. In a preferred aspect, the invention involves the use of size separation chromatography and/or filtration to separate or substantially separate soluble protein and peptide molecules from high molecular weight molecules, structures and aggregates. Thus, any matrix which

provides desired size separation (e.g., filters, chromatography supports, etc.) may be used in the invention. One of skill in the art can readily determine the appropriate matrix, pore size of the matrix, size, shape and dimensions of the matrix taking into consideration the type and size of the desired protein and peptide molecules and the cell type or cellular source. In another aspect, the invention combines such size separation/filtration with cell lysis/disruption (preferably such lysis/disruption is done when or approximately when the cellular source comes in contact with or after the cellular source is in contact with the filtration matrix). The pores or passage ways in the matrix are typically small enough to prevent passage of large molecules, structures and aggregates, but large enough to permit passage of soluble protein and peptide molecules of interest. The potential pore sizes may range from about 0.1 to about 10,000 microns in diameter, about 0.1 to about 5,000 microns in diameter, about 0.1 to about 1,000 microns in diameter, about 1 to about 500 microns in diameter, about 10 to about 500 microns in diameter, or about 25 to about 400 microns in diameter.

Please replace paragraph [0016] at pages 8-9 with the following paragraph:

In another preferred embodiment, the composition or compound that disrupts the cellular membrane or cell wall integrity may comprise one or more non-ionic detergents, including, but not limited to, N-octyl-β-D-glucopyranside, N-octyl-β-D-maltoside, ZWITTERGENT 3.14 (N-tetradecyl-sulfobetaine or 3-(tetradecyldimethylammonio)-propane-sulfonate), deoxycholate; n-Dodecanoylsucrose; n-Dodecyl-β-D-glucopyranoside; n-Dodecyl-β-D-maltoside; n-Octyl-β-D-glucopyranoside; n-Octyl-β-D-maltopyranoside; n-Octyl-β-D-thioglucopyranoside; n-Decanoylsucrose; n-Decyl-β-

D-maltopyranoside; n-Decyl-β-D-thiomaltoside; n-Heptyl-β-D-glucopyranoside; n-Heptyl-β-D-thioglucopyranoside; n-Hexyl-β-D-glucopyranoside; n-Nonyl-β-D-glucopyranoside; n-Octanoylsucrose; n-Octyl-β-D-glucopyranoside; n-Undecyl-β-D-maltoside; APO-10 (decylmethylphosphine oxide); APO-12 (dodecyldimethylphosphine oxide); Big CHAP (N,N-bis(3-glucon-amidopropyl)cholamide); Big CHAP, Deoxy (N,N-bis(3-glucon-amidopropyl)cholamide); BRIJ® 35 (polyoxyethylene lauryl alcohol); $C_{12}E_5$ (pentaethylene glycoldodecyl ether); $C_{12}E_6$ (polyoxyethylene 6 lauryl ether); $C_{12}E_8$ (polyoxyethylene 8 lauryl ether); $C_{12}E_9$ (polyoxyethylene 9 lauryl ether); Cyclohexyl-n-ethyl-β-D-maltoside; Cyclohexyl-n-hexyl-β-D-maltoside; Cyclohexyl-n-methyl-β-D-maltoside; Digitonin; ELUGENT®[[™]] (mixture of alkylglucosides); GENAPOL® C-100 (Decaoxyethylene-dodecyl-ether); GENAPOL® X-080 (octaethylene-glycol-isotridecylether); GENAPOL® X-100 (decaethylene-isotridecyl-ether); HECAMEG® (6-O-(N-heptylcarbamoyl)-methyl-alpha-D-glucopyranoside); MEGA-10 (decanoyl-N-methyl-glucamide); MEGA-8 (octanoyl-N-methyl-glucamide); MEGA-9 (nonanoyl-N-methyl-glucamide); NOGA; NP-40 (nonaethylene-glycol-octyl-phenyl-ether); PLURONIC® F-127 (polyethylene-polypropylene-glycol); TRITON® X-100 (nonaethylene-glycol-octyl-phenol-ether); TRITON® X-114 (heptaethylene-glycol-octyl-phenylether; condensation product of octylphenol with seven or eight moles of ethylene oxyde; polyoxyalkylenated alkylphenol); TWEEN® 20 (polyoxyethylene-sorbitane-monolaurate); or TWEEN® 80 (polyoxyethylene-sorbitane-monooleate). Additionally, an ionic detergent can be used with the methods of the invention, including, but not limited to BATC (8,8-bis(4-aminophenyl) tricyclo(5,2,1,0.sup.2,6) decane), Cetyltrimethylammonium Bromide, Chenodeoxycholic Acid, Cholic Acid, Deoxycholic Acid, Glycocholic Acid, Glycodeoxycholic Acid, Glycolithocholic Acid,

Lauroylsarcosine, Taurochenodeoxycholic Acid, Taurocholic Acid, Taurodehydrocholic Acid, Taurolithocholic Acid, Tauroursodeoxycholic Acid, and TOPPA (1,1',2,2'-tetraoleoyl pyro phosphatidic acid). Zwitterionic detergents can also be used with the compositions and methods of the invention, including, but not limited to, amidosulfobetaines, CHAPS (3-((cholamidopropyl)-dimethylammonio)-1-propane sulfonate), CHAPSO (3-((cholamidopropyl)-dimethylammonio)-2-hydroxy-1-propane sulfonate), carboxybetaines, and methylbetaines. In addition one or more enzymes such as zymolyase, lyticase, lysozyme or lysostaphin; one or more inorganic salts such as sodium chloride, potassium chloride, or lithium chloride; one or more acids and/or bases or buffering agents (e.g., to increase or reduce pH); or any other compound or enzyme which may assist in the disruption of the integrity of (i.e., lyses or causes the formation of pores in) the cell membrane and/or cell walls (e.g., polymixin B) can be used. In another aspect, the composition may comprise one or more compounds or enzymes to degrade, destroy or remove unwanted components or contaminants (e.g., ribonucleases (RNases), DNases, and nucleases (e.g. endonucleases and exonucleases) to remove or destroy or degrade undesired nucleic acid molecules (e.g., DNA or RNA) released from the cellular source). If soluble protein in its native conformation is desired then a non-denaturing detergent should be used. In one particularly preferred aspect, the cell lysis/disruption composition may be adsorbed onto or complexed with or associated with the matrix prior to applying the one or more cells or cellular source to the matrix. In a preferred aspect, the composition is dried in or on the matrix. Thus, in a preferred aspect, the matrix comprises a cell lysis/disruption compound or composition. In this aspect, the cell disruption/lysis may occur when or about the same time the cells come into contact with the composition containing matrix. In another aspect, the composition

is added after the cells are added to (e.g., bound to or associated with) the matrix. In yet another aspect, the composition is added to the cells prior to adding the cells to the matrix. In this aspect, the composition may be formulated to weaken the cell membrane/cell wall such that the cells will substantially disrupt/lyse when contacted with the matrix. Alternatively, the composition will substantially lyse/disrupt the cells before addition to the matrix.

Please replace paragraph [0018] at page 10 with the following paragraph:

In another preferred embodiment of the invention, after the soluble protein and peptide molecules have been eluted or removed from the matrix, the matrix, containing the insoluble materials (e.g. membrane fragments and/or inclusion bodies) is contacted with a second elution/disruption reagent (e.g. 6M Urea) which causes the disruption of the insoluble materials (membrane fragments and/or inclusion bodies), and the solubilization of the constituent proteins. These liberated protein or peptide molecules can then be eluted or substantially removed from the matrix. Such elution or removal of the soluble protein and peptide molecules, with or without the addition of an aqueous solution, may be facilitated by centrifugation, gravity, vacuum, pressure, etc., which provides flow of the desired protein or peptide sample from the matrix. Appropriate compositions included in the second elution buffer include compositions capable of disrupting and solubilizing the protein or peptide molecules present in an inclusion body or membrane fragment as appropriate. Appropriate compositions include, but are not limited to, urea, guanadinium chloride, detergents, ~~chaeotropic~~ chaotropic agents, salts, and the like. In another aspect of the invention, cell lysis/disruption or

disruption/solubilization of insoluble material can be accomplished in one step, preferably with one composition or reagent that serves both functions. Such compositions may comprise, but are not limited to, urea, guanadinium chloride, ionic or non-ionic detergents, and the like.

Please replace paragraph [0024] at page 12 with the following paragraph:

Preferred cellular sources, solid matrices, and lysis/disrupting/-permeabilization compounds for use in the compositions of the invention include those described and used in the methods of the present invention. In a preferred composition of the invention, an effective amount of the compound that disrupts the integrity of the cellular membrane and/or cell wall is adsorbed onto or complexed with or associated with the matrix, for example by ionic, hydrophobic, or covalent or non-covalent attachment of the cell membrane/cell wall disrupting compound to the matrix material. In one embodiment, such compound is dried in or on the matrix. While some commercially available matrixes have small amounts of TRITON® detergent incorporated into the fibers for manufacturing purposes, the detergent is not present in an amount large enough to cause substantial lysis/disruption/permeabilization of cells. Thus, according to the present invention, at least one additional cell lysis/disruption/permeabilization composition typically is added or used according to the methods of the invention. The compositions of the invention are useful in isolating a variety of proteins and peptide molecules, particularly those described herein.

Please replace paragraph [0063] at pages 28-29 with the following paragraph:

In one preferred embodiment, an effective amount of the composition that disrupts the cellular membrane/cell wall integrity that is applied to the matrix, or that is pre-adsorbed onto the matrix, may comprise one or more detergents, which may be a non-ionic detergent, including, but not limited to, N-octyl-β-D-glucopyranside, N-octyl-β-D-maltoside, ZWITTERGENT 3.14 (N-tetradecyl-sulfobetaine or 3-(tetradecyldimethylammonio)-propane-sulfonate), deoxycholate; n-Dodecanoylsucrose; n-Dodecyl-β-D-glucopyranoside; n-Dodecyl-β-D-maltoside; n-Octyl-β-D-glucopyranoside; n-Octyl-β-D-maltopyranoside; n-Octyl-β-D-thioglucopyranoside; n-Decanoylsucrose; n-Decyl-β-D-maltopyranoside; n-Decyl-β-D-thiomaltoside; n-Heptyl-β-D-glucopyranoside; n-Heptyl-β-D-thioglucopyranoside; n-Hexyl-β-D-glucopyranoside; n-Nonyl-β-D-glucopyranoside; n-Octanoylsucrose; n-Octyl-β-D-glucopyranoside; n-Undecyl-β-D-maltoside; APO-10 (decylmethylphosphine oxide); APO-12 (dodecyldimethylphosphine oxide); Big CHAP (N,N-bis(3-glucon-amidopropyl)cholamide); Big CHAP, Deoxy (N,N-bis(3-glucon-amidopropyl)cholamide); BRIJ® 35 (polyoxyethylene lauryl alcohol); $C_{12}E_5$ (pentaethylene glycoldodecyl ether); $C_{12}E_6$ (polyoxyethylene 6 lauryl ether); $C_{12}E_8$ (polyoxyethylene 8 lauryl ether); $C_{12}E_9$ (polyoxyethylene 9 lauryl ether); Cyclohexyl-n-ethyl-β-D-maltoside; Cyclohexyl-n-hexyl-β-D-maltoside; Cyclohexyl-n-methyl-β-D-maltoside;Digitonin; ELUGENT®[[™]] (mixture of alkylglucosides); GENAPOL® C-100 (Decaoxyethylene-dodecyl-ether); GENAPOL® X-080 (octaethylene-glycol-isotridecylether); GENAPOL® X-100 (decaethylene-isotridecyl-ether); HECAMEG® (6-

O-(N-heptylcarbamoyl)-methyl-alpha-D-glucopyranoside); MEGA-10 (decanoyl-N-methyl-glucamide); MEGA-8 (octanoyl-N-methyl-glucamide); MEGA-9 (nonanoyl-N-methyl-glucamide); NOGA; NP-40 (nonaethylene-glycol-octyl-phenyl-ether); PLURONIC® F-127 (polyethylene-polypropylene-glycol); TRITON® X-100 (nonaethylene-glycol-octyl-phenol-ether); TRITON® X-114 (heptaethylene-glycol-octyl-phenylether; condensation product of octylphenol with seven or eight moles of ethylene oxyde; polyoxyalkylenated alkylphenol); TWEEN® 20 (polyoxyethylene-sorbitane-monolaurate); or TWEEN® 80 (polyoxyethylene-sorbitane-monooleate). Additionally, the detergent may be an ionic detergent, including, but not limited to, BATC, Cetyltrimethylammonium Bromide, Chenodeoxycholic Acid, Cholic Acid, Deoxycholic Acid, Glycocholic Acid, Glycodeoxycholic Acid, Glycolithocholic Acid, Lauroylsarcosine, Taurochenodeoxycholic Acid, Taurocholic Acid, Taurodehydrocholic Acid, Taurolithocholic Acid, Tauroursodeoxycholic Acid, and TOPPA (1,1',2,2'-tetraoleoyl pyro phosphatidic acid). Zwitterionic detergents can also be used with the compositions and methods of the invention, including, but not limited to, amidosulfobetaines, CHAPS (3-((cholamidopropyl)-dimethylammonio)-1-propane sulfonate), CHAPSO (3-((cholamidopropyl)-dimethylammonio)-2-hydroxy-1-propane sulfonate), carboxybetaines, and methylbetaines

Please replace paragraph [0064] at pages 29-30 with the following paragraph:

The concentration of the detergent may be from about 0.01 to 10 % by weight, 0.01 to 5% by weight, 0.01 to 4% by weight, 0.01 to 3% by weight, 0.01 to 2.5% by weight, 0.1 to 10% by weight, 0.1 to 5% by weight, 0.1 to 4% by weight, 0.1 to 3% by

weight, 0.1 to 2.5% by weight, 0.5 to 10% by weight, 0.5 to 5% by weight, 0.5 to 4% by weight, 0.5 to 3 % by weight, 0.5 to 2.5% by weight, 1.0 to 10% by weight, 1.0 to 5% by weight, 1.0 to 4% by weight, 1.0 to 3 % by weight or 1.0 to 2.5% by weight. Most preferably the detergent concentration is 2.5%. In addition, one or more enzymes such as lysozyme, lyticase, zymolyase, neuraminidase, streptolysin, cellulysin, mutanolysin, chitinase, glucalase or lysostaphin may be used, at a concentration of about 0.1 to 5 mg/ml; one or more inorganic salts such as sodium chloride, potassium chloride, magnesium chloride, calcium chloride, lithium chloride, or praseodymium chloride at a concentration of about 1 mM to 5M; or any other compound which disrupts the integrity of (i.e., lyses or causes the formation of pores in) the membrane and/or cell wall of the cellular source of protein and peptide molecules (e.g., polymixin B), or combinations of the foregoing may be used. The compositions may also comprise other components, such as protease inhibitors (e.g., phenylmethylsulfonyl fluoride, trypsin inhibitor, aprotinin, pepstatin A), reducing reagents (e.g., 2-mercaptoethanol and dithiothreitil) at concentrations of 0.1 to 10 mM, chelating agents (e.g., disodium ethylenediaminetetraacetic acid (Na_2EDTA), EGTA, CDTA, most preferably at a concentration of about 1 mM or less) and/or one or more ribonucleases (RNase A, T1, T2, and the like) at concentrations ranging from 1 to 400 <g/ml, or any combination of the foregoing. DNase I concentrations may range from 1 to 100 units (10,000 units/mg). In one preferred embodiment, the composition provides for the disruption of the cell membrane or cell wall integrity without substantially perturbing the native conformation or function of the desired proteins and peptides, so that a protein or peptide having the native conformation, or substantially the native conformation may be collected. However, if the native structure of the protein or peptide is not required, then no

limitation on the lysis/disruption reagent is required. The lysis/disruption compositions preferably comprises less than 10% cell lysis/disruption/permeabilization composition, more preferably, less than 5% cell lysis/disruption/permeabilization composition and most preferably, less than 3% cell lysis/disruption/permeabilization composition. A most preferred composition comprises 2.5% ELUGENT®[[™]] (mixture of alkylglucosides), Calbiochem Corporation (San Diego, CA). In other embodiments of the invention, the ELUGENT®[[™]] (mixture of alkylglucosides) concentration may range from about 0.01 to 10 % by weight, 0.01 to 5% by weight, 0.01 to 4% by weight, 0.01 to 3% by weight, 0.01 to 2.5% by weight, 0.1 to 10% by weight, 0.1 to 5% by weight, 0.1 to 4% by weight, 0.1 to 3% by weight, 0.1 to 2.5% by weight, 0.5 to 10% by weight, 0.5 to 5% by weight, 0.5 to 4% by weight, 0.5 to 3 % by weight, 0.5 to 2.5% by weight, 1.0 to 10% by weight, 1.0 to 5% by weight, 1.0 to 4% by weight, 1.0 to 3 % by weight or 1.0 to 2.5% by weight. Desired concentrations and combinations of the active ingredients of the lysis/disruption compositions may be readily determined by those skilled in the art. In another aspect of the invention, cell lysis/disruption and disruption/solubilization of insoluble material can be accomplished with one composition or reagent that serves both functions.

Please replace paragraph [0101] at pages 45-46 with the following paragraph:

Lysis matrix/filter matrix is a simpler and more powerful protein extraction procedure than commercially available products, such as ~~BugBuster~~[[™]]BUG BUSTER® (detergent containing Complete Protease Inhibitor Cocktain Tablets (Roche))and B-PER® (lysis buffer containing mild nonionic detergent in 20 mM Tris

HCl, pH 7.5). Since genomic DNA does not appear in samples from Lysis matrix/filter matrix, there are no sample viscosity problems to overcome with separate digestion with ~~Benzonase~~BENZONASE® Nuclease (recombinant endonuclease) as is the case with ~~BugBuster~~[[™]] BUG BUSTER® (detergent containing Complete Protease Inhibitor Cocktain Tablets (Roche)). In addition, maintaining most of the nucleic acids within the cell when Permeabilization Buffer is used, provides a lower background for enzymes used in molecular biological procedures. Furthermore, using Lysis matrix/filter matrix retains cell membranes, separating them and many biomolecules away from the soluble extracted proteins.

Please replace paragraph [0128] age page 55 with the following paragraph:

Protein Extraction by 96 well Lysis matrix/filter matrix. Duplicate 200 ul aliquots of culture were applied directly to the filter surface of the 96-Well Lysis matrix/filter matrix. 100 ul of Lysis Buffer (150mM sodium phosphate pH 8.0, 300mM NaCl, 2%(v/v) ELUGENT®[[™]] (mixture of alkylglucosides), 1.5%(v/v) Triton X-100 (nonaethylene-glycol-octyl-phenol-ether), 0.025mg/ml lysozyme) were added to both filters. Incubation continued for 10min at room temperature, then the 96 Well Lysis matrix/filter matrix plate was aligned on top of a 96-well, 650 ul receiver plate (Cat. No. p9605, Labnet International) and centrifuged 5 min at 1000 x *g* in swing bucket rotor. Soluble protein was collected in the receiver plate and the inclusion bodies were trapped in the matrix. The matrix was then washed with 500 ul of ddH_2O and centrifuged for 5 min. at 1000 x *g*. The wash was discarded. The 96 Well Lysis matrix/filter matrix plate was aligned on top of another 96-well, 650 ul receiver plate (Cat. No. p9605, Labnet

International). 300 ul of Insoluble Buffer (150mM sodium phosphate pH 8.0, 8M urea, 300mM NaCl) was added to the filter and incubated for 10min at room temperature. The plate was then centrifuged at 1000 x *g* for 5 min in a swinging bucket rotor, and the solubilized protein was collected in the collection plate.

Please replace paragraph [0132] at page 56 with the following paragraph:

Protein Extraction by 96 well Lysis matrix/filter matrix. Duplicate 200 µl aliquots of induced culture were applied directly to the filter surface of the 96-Well Lysis matrix/filter matrix. 100 µl of Lysis Buffer (150 mM sodium phosphate pH 8.0,.300 mM NaCl, 2%(v/v) ELUGENT®[[™]] (mixture of alkylglucosides), 1.5%(v/v) Triton X-100 (nonaethylene-glycol-octyl-phenol-ether), 0.025 mg/ml lysozyme) were added to both filters. Incubation continued for 10 min at room temperature, then the 96 Well Lysis matrix/filter matrix plate was aligned on top of a 96-well, 650 µl-receiver plate (Cat. No. p9605, Labnet International) and centrifuged 5 min at 1000 x *g* in swing bucket rotor. Soluble protein was collected in the receiver plate.

In the Abstract

Please replace the abstract at page 65 with the following abstract:

The present invention relates generally to compositions, methods and kits for use in extracting and isolating protein or peptide molecules. More specifically, the invention relates to such compositions, methods and kits that are useful in the isolation of protein or peptide molecules from cells. The compositions, methods and kits of the invention are suitable for isolating a variety of forms of protein or peptide molecules from cells. The compositions, methods and kits of the invention are particularly well-suited for rapid isolation of recombinant proteins or peptide molecules.

A replacement abstract is also provided herewith on a separate sheet.